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Microalgal Fuel Production Processes
Analysis of Lipid Extraction and Conversion Methods

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ABSTRACT

Microalgae are unique photosynthetic organisms that are known to accumulate storage lipids in large quantities and thrive in saline waters. Before these storage lipids can be used they must first be extracted from the microalgae and converted into usable fuel. Transesterification of lipids produces fatty acid methyl esters that can be used as a diesel fuel substitute.

Three solvents, 1-butanol, ethanol, and hexane/2-propanol were evaluated for extraction efficiency of microalgal lipids. Type of catalyst, concentration of catalyst, time of reaction, temperature of reaction, and quality of lipid were examined as variables for transesterification. The most efficient solvent of the three for extraction was 1-butanol (90 % efficiency), followed by hexane/2-propanol and ethanol. Optimal yield of fatty acid methyl esters was obtained using 0.6 N hydrochloric acid in methanol for 0.1 h at 70° C.

MICROALGAL FUEL PRODUCTION PROCESSES: ANALYSIS OF EXTRACTION AND CONVERSION METHODS

INTRODUCTION

World-wide energy shortages and the oil embargo of the 1970's forced many nations to evaluate energy sources and supplies. Energy from biomass is an attractive solution because it is renewable. One of the goals of U.S. Department of Energy and the Solar Energy Research Institute is to develop a technology base for the large scale production of lipid-producing microalgae and conversion of microalgal lipids into substitutes for traditional fuels.

Neenan et al. (1986) analyzed the production of gasoline and diesel fuel substitutes using mass culture of microalgae. Large scale raceways of microalgae could yield 150-400 barrels of oil/acre/year. Two methods of conversion have been proposed (Neenan, et al., 1986): (1) transesterification for ester fuel production and (2) zeolite catalysis for gasoline production. While production of methyl esters from seed crops has been studied extensively, research on methyl esters from microalgal lipids is limited. This paper will report the results of our study which examined several solvent systems for lipid extraction from microalgae and identified the impact of major variables in transesterification of microalgal lipids.

MATERIALS AND METHODS

BIOMASS PRODUCTION

Biomass was produced for lipid extraction and conversion work at two locations - the Solar Energy Research Institute Field Test Laboratory Building (FTLB), and the Solar Energy Research Institute Outdoor Test Facility (OTF) in Roswell, New Mexico (Johnson et al. 1987). At the FTLB, the diatom Chaetoceros myelleri (SERI designation CHAET9, Johansen et al. 1987) was grown in five 1.4 m² ponds in a greenhouse. The ponds were filled to a depth of 20 cm with SERI Type II/10 water as a growth medium (Barclay et al., 1986). The ponds were heated to maintain a minimum temperature of 19° C. High pressure sodium lamps (Applied Hydroponics, San Rafael, CA) were used to continuously maintain a minimum light intensity of 400 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Growth medium was pH-controlled at an upper limit of 9.5, by carbon dioxide injection. Ambient air and water temperature and light intensity were monitored, and pH was controlled by an Apple computer using a Strawberry Tree data acquisition card (Sunnyvale, CA). Growth rates and nutrient status were monitored daily. The maximum growth rate obtained was 28 g·m⁻²·d⁻¹ of ash free dry weight.

At the OTF, the alga Monoraphidium minutum (SERI designation MONOR2, Barclay et al., 1986) was grown in three 3.0 m² ponds outdoors. The ponds were filled to a depth of 15 cm using on-site saline ground water as a growth medium. The ponds were not heated and received only natural illumination. pH was controlled to an upper limit of 9.5.

HARVESTING

At the FTLB, biomass was harvested by pressure filtration. A filter press (Star Systems, Timmonsville, SC) with twenty plates and frames (30 cm in diameter) with 5 μm pore size paper filters was used to harvest the algae.

Growth medium and cells were pumped through the press at a rate of 800 L·h⁻¹. Filtrate (growth medium) was returned to the ponds, through four cycles of growth and harvest. The biomass was collected on the paper filters. Biomass was concentrated from an initial concentration in the ponds of 0.5 % solids, to a final concentration of 8.0 % solids.

At the OTF, polymer flocculation (Weissman, unpublished) was the harvest method chosen. Biomass was concentrated from an initial density of 0.5 % solids to a final density of 4.0 % solids.

EXTRACTION

Lipid extractions were performed with a variety of solvents to determine the extraction efficiency compared with a control method. Three replicate trials of each solvent extraction were performed on *C. muelleri*. All extractions were batch runs, timed for 90 min. Temperature was varied, depending on the boiling point of the solvent. Solvent to biomass ratio in all trials was three:one (see below). All biomass percentages are expressed on a wet weight per volume basis.

The solvents used were 1-butanol, ethanol, and a mixture of hexane and 2-propanol. The hexane/2-propanol solvent system was 40 % hexane and 60 % 2-propanol, by volume. The control method was run in triplicate. This was a five step water/methanol/chloroform extraction (Roessler 1986).

Harvested biomass (8.0 % solids) was concentrated by centrifugation to 15.0 % solids, to reduce handling difficulty and volume of solvent needed. A 400 g sample of concentrated biomass was mixed with 1200 g of solvent. The mixture was heated to near boiling and maintained at that temperature for 90 min. The mixture was well-agitated during the extraction, then vacuum-filtered through a glass fiber filter to separate the lipid and solvent from the solid residue. Next, the solvent was removed by distillation. The lipid was further purified by a chloroform/methanol/water phase separation. A second distillation was run to remove the solvent from the purified lipid. The extracted lipid was then weighed. Purity was quantitatively determined (Delamas et al. 1984) using a thin layer chromatography scanner with flame ionization detector (Iatron Laboratories, Inc., Tokyo). The resulting yield of the pure lipid was compared with the control yield, to arrive at the relative efficiency for each extraction.

CONVERSION OF MICROALGAL LIPID

A Plackett-Burman screening design (Murray 1984) was used to screen major variables that affect transesterification. Variables examined were type of catalyst (hydrochloric acid-methanol and sodium hydroxide-methanol), concentration of catalyst (0.12 N and 0.6 N), time of reaction (0.1 and 3.0 h), temperature of reaction (20° and 70° C), and type of feedstock used, (lipid extracted from *M. minutum* and *C. muelleri*). A sample of 250 mg of the designated lipid was weighed into a teflon screw-cap tube, 5 mL of the catalyst-methanol solution was added and incubated at the appropriate time and temperature. The samples were agitated every 10 min. Fatty acid methyl esters (FAME) were extracted by addition of 5 mL of petroleum ether followed by vortexing and centrifugation. The top layer was drawn off, evaporated at 50° C and weighed. The dependent variable in the regression model was called YIELD, which was the amount of FAMEs produced by the individual treatment. FAME identification was done using the Iatroscan. The efficiency of methylation of known algal lipids was determined using individual lipid

standards, at conditions chosen using the Plackett-Burman screening design.

RESULTS

EXTRACTION

For C. muelleri, the highest and most consistent extraction efficiency and lipid purity was obtained by using 1-butanol. Average extraction efficiency for the butanol trials was 90 % (w/w), with a relative standard deviation (RSD) of 3 %. The fraction of pure lipid in total extractables was 0.94.

Ethanol (95 % ethanol, 5 % propanol, v/v) gave an average lipid extraction efficiency of 73 % (RSD of 13 %), and a pure lipid fraction of 0.90. The hexane/2-propanol solvent system gave an extraction efficiency of 78 % (RSD of 26 %), and a pure lipid fraction of 0.90 (See Table 1). All efficiency and purity results are averages of three "large scale" (150 to 400 g of biomass feedstock) extraction trials. Each large scale trial was run with three small scale (1.0 to 1.5 g of biomass) controls to determine theoretical 100 % yields.

One large scale extraction was run on the alga M. minutum. M. minutum was grown outdoors at the Roswell, New Mexico Test Facility. The solvent used for this extraction was 1-butanol. Extraction efficiency was 81 %, with a pure lipid fraction of 0.96.

CONVERSION

Variables examined in the Plackett-Burman screening design were time, temperature, type of catalyst, concentration of catalyst, and type of lipid used. Results indicate (Table 2) that type of catalyst was the most important variable. Time, concentration of catalyst, lipid type, and temperature of reaction were not as important. The explained coefficient of determination (R^2) was 74 %. Type of catalyst alone could explain over 55 % of the variation in the model. The maximum yield of FAMES was achieved at the following conditions: 0.6 N hydrochloric acid-methanol catalyst, for 0.1 h, at 70° C, using the extracted lipid from C. muelleri. Conversion yields of known algal lipids (Table 3) indicate that fatty acids had the highest yield (93 %), while monogalactosyl diglyceride had the lowest yield, 46 %.

DISCUSSION

EXTRACTION

Solvents were selected for analysis based on several criteria. These were low carcinogenicity, ease and safety of handling, volatility, availability and cost, and previous research. Ethanol, 1-butanol, and hexane/2-propanol appeared to be the most promising solvents.

All solvents were fairly effective at extracting a pure lipid product, at reasonably high efficiencies, with 1-butanol obviously being the best of the three. Butanol extractions were also the most consistent from trial to trial, in efficiency and purity. Although all solvents were relatively easy to handle, the consistency of the butanol results indicates a lower sensitivity to changes (or errors) in extraction procedure. This may be a considerable benefit as the process is scaled up.

The source of biomass (C. muelleri vs M. minutum) does not appear to significantly affect extraction efficiency. While only one extraction trial with M. minutum was run, its efficiency was not significantly different from the C. muelleri extractions.

All conversion studies were run with lipid extracted with 1-butanol. While all three solvents provided comparably pure lipid, downstream effects (conversion efficiency) were not investigated as a function of extraction method. Future work may include studies of the downstream effects of extraction solvent choice.

CONVERSION

Transesterification of seed oils has been well documented (Engler 1983). Freedman et al. (1984) optimized the variables for ester formation from triacylglycerols. The feedstock should have less than 0.5 % free fatty acids, 1 % sodium hydroxide-methanol should be used in a 6:1 molar ratio of alcohol to oil, and the mixture should be refluxed for 1 h at 60° C. Freedman found that yields from seed oils using acid catalyst were lower, the reaction took longer, and higher temperatures were required when hydroxide catalyst was used. Freedman (1984) and Nye (1983) recommended acid catalyst when starting materials are low grade or have a high concentration of free fatty acids; the fatty acids would deactivate an alkaline catalyst. The concentration of free fatty acids in the lipid from C. muelleri and M. minutum was 24.6 %; thus an acid catalyst would be more effective than a base catalyst. The high confidence coefficient for the hydrochloric acid-methanol catalyst over the sodium hydroxide catalyst confirms this. The negative coefficient for time suggests that longer incubation times are deleterious to FAME yields. Temperature was not significant. A possible explanation might be related to the high concentration of free fatty acids in the algal lipid. The lower conversion yields from the phosphatidyl choline, phosphatidyl glycerol, mono- and digalactosyl diglyceride esters can be explained by the substitution of fatty acid groups by carbohydrate and phosphate groups.

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TABLE 1. Efficiency of lipid extraction from C. muelleri for several different solvents. All extractions were heated to near-boiling and run batchwise for 90 min. All results below are averages of three trials, with coefficients of variation in parentheses. Gross efficiency is actual lipid yield divided by theoretical yield from control extractions. Net efficiency accounts for the purity of extracted lipid. Purity is determined by thin layer chromatography (IATROSCAN).

SOLVENT	GROSS EFFICIENCY	PURITY	NET EFFICIENCY
1-butanol	94 % (3)	0.94 (3)	90 % (3)
ethanol	82 % (12)	0.90 (4)	73 % (13)
hexane/2-propanol	87 % (25)	0.90 (6)	78 % (26)

Note: One extraction trial was run with M. minutum feedstock and 1-butanol solvent. The results were gross efficiency of 84 %, purity fraction of 0.96, and net efficiency of 81 %.

TABLE 2. Regression coefficients for yield of fatty acid methyl esters using a Plackett-Burman screening design. Lipids from C. muelleri and M. minutum. were transesterified at different combinations of time, temperature, catalyst, and catalyst concentration. Type of catalyst was identified as the most important, having the largest coefficient of determination.

Coefficient	Term	Std. Error	Confidence Coef.
8.2	Lipid	11.1	49.4 %
0.3	Temp	0.4	49.9 %
-10.7	Time	7.7	77.9 %
39.9	Catalyst	11.1	98.5 %
5.2	Conc	4.5	69.5 %

TABLE 3. Yield of fatty acid methyl esters (FAME) from standards representing lipids found in algae. All yields are the mean of two samples of lipid standards. Reactions took place using 5 mL of 0.6 N hydrochloric acid in methanol at 70° C for 1.5 h. Yield is based on mass of FAME produced divided by mass of starting material.

LIPID	% YIELD
Palmitic acid	93.4%
Triolein	87.6%
Phosphatidyl glycerol	54.4%
Phosphatidyl choline	65.1%
Monogalactosyl diglyceride	46.6%
Digalactosyl diglyceride	55.8%

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